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Marriage and motherhood are associated with lower testosterone concentrations in women.

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Abstract:

Testosterone has been hypothesized to modulate the trade-off between mating and parenting effort in males. Indeed, evidence from humans and other pair-bonded species suggests that fathers and men in committed relationships have lower testosterone levels than single men and men with no children. To date, only one published study has examined testosterone in relation to motherhood, finding that mothers of young children have lower testosterone than non-mothers. Here, we examine this question in 195 reproductive-age Norwegian women. Testosterone was measured in morning serum samples taken during the early follicular phase of the menstrual cycle, and marital and maternal status were assessed by questionnaire. Mothers of young children (age ≤ 3) had 14% lower testosterone than childless women and 19% lower testosterone than women who only had children over age 3. Among mothers, age of the youngest child strongly predicted testosterone levels. There was a trend towards lower testosterone among married women compared to unmarried women. All analyses controlled for body mass index (BMI), age, type of testosterone assay, and time of serum sample collection. This is the first study to look at testosterone concentrations in relation to marriage and motherhood in Western women, and it suggests that testosterone may differ with marital and maternal status in women, providing further corroboration of previous findings in both sexes.

Keywords: marriage; motherhood; testosterone; endocrinology

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Introduction:

In males, testosterone plays a key role in modulating both mating effort and paternal investment. Physiologically, testosterone facilitates reproductive effort by promoting spermatogenesis and supporting the development of sexually dimorphic traits such as upper body muscle mass and increased stature (Bribiescas, 2001). Testosterone also contributes to reproductive effort behaviorally by promoting male-male competition and mate-seeking behaviors (Archer, 2006). The “challenge hypothesis”, which emerged from avian research, posits that in species with biparental care, testosterone levels rise in contexts of male-male competition (particularly related to mating) and then decrease to facilitate care of young, dependent offspring (De Ridder et al., 2000; Nunes et al., 2000; Peters et al., 2002; Reburn and Wynne-Edwards, 1999; Wingfield et al., 1990; Ziegler, 2000). In humans, cross-sectional data suggests that testosterone levels are higher in: (1) unmarried men versus married men (Booth and Dabbs, 1993; Gray et al., 2002; Kuzawa et al., 2009); (2) uncommitted single men versus single men in committed relationships (Burnham et al., 2003; McIntyre et al., 2006; Sakaguchi et al., 2006; van Anders and Watson, 2006); and (3) non-fathers versus fathers (Gray et al., 2006b; Kuzawa et al., 2009; Muller et al., 2009). Longitudinal work supports this hypothesis as well. One cohort study found that testosterone levels were highest prior to marriage, declined during marriage, and then rose again if divorce occurred (Mazur and Michalek, 1998). Similarly, among single men without children, those who partnered and became fathers during a 4.5 year follow-up period had greater testosterone declines than those who remained single and childless (Gettler et al., 2011a).

Despite great interest in testosterone, marriage, and fatherhood in males, virtually no research has investigated this question in females and the role of testosterone in modulating female mating effort and maternal behaviors is poorly understood. Although testosterone clearly plays

97 a critical role in *some* components of female mating behavior, such as libido (Braunstein et al.,
98 2005; van Anders et al., 2007), its contribution to other aspects of female mating and parenting
99 effort are less apparent. Traditional evolutionary theory holds that intra-sexual mating
100 competition is weak in human females and thus if testosterone primarily modulates intra-sexual
101 mating competition, there should be little association with mating and maternal behavior
102 (Bateman, 1948; Trivers, 1972). Nevertheless, research suggests that testosterone may be
103 associated with mating behaviors in females of some species. In numerous avian species,
104 female testosterone levels vary across the breeding cycle, typically peaking during ovulation
105 and/or prior to the egg laying period, concurrent with the acquisition of mates and territory
106 (Cristol and Johnsen, 1994 ; Gill et al., 2007; Ketterson et al., 2005; Osorno et al., 2010). In
107 humans, several studies have found positive associations between testosterone and female
108 aggression or competition, however it is unclear whether these findings are relevant within a
109 mating context (Edwards and Kurlander, 2010; Harris et al., 1996). In fact, the limited research
110 on female testosterone levels and mating status to date has been inconsistent. While some
111 studies have found that among heterosexual women, being in a committed, monogamous
112 relationship is associated with lower testosterone concentrations (Edelstein et al., 2011; van
113 Anders and Watson, 2006), others have failed to find a relationship (Hooper et al., 2011).

114

115 Similarly, the research on testosterone and motherhood is in its infancy. Although there is some
116 evidence that in marmosets, elevated testosterone is associated with decreased care-giving
117 among mothers (Fite et al., 2005), to our knowledge, only one published study has examined
118 testosterone, pair-bonding, and motherhood in humans (Kuzawa et al., 2010). In a cohort of
119 Filipino women, waking salivary testosterone levels were lower in pair-bonded versus single
120 women, and in mothers versus non-mothers. Mothers of young children, moreover, had
121 significantly lower testosterone levels than mothers of older children, and only motherhood
122 remained a significant predictor of testosterone levels in multiple regression models. While the

123 results are suggestive, the sample size (n=67) was small and population demographics skewed
124 heavily towards very young mothers and included few married women without children.
125 Furthermore, the study included women who were breast-feeding and/or using hormonal
126 contraception, and testosterone levels were assayed from sample taken at different points in the
127 menstrual cycle. Given these limitations, we attempted to replicate their study using stricter
128 inclusion criteria in a second, demographically different population.

129

130 To this end, we used data from a Norwegian cohort study to test the following primary
131 predictions: (1) testosterone levels are lower in married women than unmarried women; and (2)
132 testosterone levels are lower in women with at least one child under age 3 than in women who
133 do not have a child under 3 (*i.e.* are either nulliparous or have only older children). Among the
134 mothers, furthermore, we predicted that the age of the youngest child and total parity would both
135 be negatively associated with testosterone levels.

136

137 **Materials and methods:**

138 *Study Population:*

139 206 women participated in the Energy Balance and Breast Cancer Aspects (EBBA-I) study,
140 based in Tromsø, Norway from 2000-2002. Participants were age 25-35 with self-described
141 regular menstrual cycles and no use of hormonal contraceptives within the past six months.
142 Women who had been pregnant or had breast-fed within the previous six months were excluded
143 from participating in EBBA-I, as were women with known histories of infertility, gynecological
144 disorders, or chronic illness. Women participated for one menstrual cycle and received 1000
145 Norwegian kroner (approximately \$160 USD) on average to cover expenses associated with
146 participation. Human subject approvals were obtained from Institutional Review Boards at all
147 participating institutions. The study subjects and protocol have been previously described in
148 greater detail (Furberg et al., 2005). For the current primary analyses, any woman who had

149 complete data on testosterone level, marital status, motherhood, age, height, and weight was
150 included in the analysis (n=195). The 11 excluded women lacked serum data (n=6),
151 questionnaire data (n=5), or both (n=2). Only mothers were included in secondary analyses
152 (n=84).

153

154 *Testosterone concentrations:*

155 Fasting morning serum samples were collected during the early follicular phase (day 1-2) of the
156 menstrual cycle and sample collection time was recorded to control for diurnal variation (Table
157 1). Fresh serum was allowed to rest for an hour after which it was centrifuged and immediately
158 assayed at the University of Northern Norway (UNN) Department of Clinical Chemistry
159 laboratory. Samples collected in the first few months of the study were assayed using Immuno1
160 from Bayer Diagnostics, after which the laboratory switched to the Elecsys 2010 assay from
161 Roche Diagnostics. For both assays, the limit of detection was 1.0 nmol/L, which is high
162 compared to those afforded by more recent female testosterone assay technologies. Average
163 intra-assay variability was less than 5% for Immuno1, and inter-assay variability ranged from
164 4.0% for low pools to 3.7% for high pools. Average inter-assay variability was 6.5% for Elecsys
165 2010 and inter-assay variability ranged from 5.7% for low pools to 2.6% for high pools.

166

167 *Motherhood and marriage:*

168

169 At baseline, women completed a questionnaire which included marital status (single,
170 married/living as married, widowed, divorced/separated, or other). Women who were divorced
171 or separated (n=6) were grouped with single women for these analyses. No women reported
172 being widowed or "other". For brevity, in this paper, we refer to the women who reported being
173 "married/living as married" as simply, "married". Subjects also reported whether they had
174 children or not. The birthdates of those children were collected during follow-up interviews in fall
175 2004 and using that information, motherhood status, parity, and age of youngest child at

176 baseline were calculated. From this data, two additional variables were derived: (1) youngest
177 child \leq age 3 at baseline; (2) youngest child $>$ age 3 at baseline. Age three was chosen as a
178 cutoff for two reasons: (1) it tends to mark a transition away from complete physical dependence
179 on parental care and increased self-sufficiency; and (2) the sample sizes of the two resulting
180 groups were sufficient to make inferences about the relationship between maternal testosterone
181 and motherhood.

182
183 *Assessment of additional covariates:*
184

185 Based on previous work in other populations suggesting that energetic factors such as energy
186 intake (Hainer et al., 2001), physical activity (Enea et al., 2011), and sleep (Andersen et al.,
187 2011) may affect testosterone levels in women in some contexts, we included several additional
188 variables in our secondary analyses. In the questionnaires, subjects reported typical hours of
189 sleep per night as well as typical physical activity level in the past year (on a scale of 1-4; 1=
190 sedentary or low activity, 2=moderate activities at least 4 hours per week, 3= hard activities to
191 keep fit for at least 4 hours per week; 4=hard training or exercise for competition several times
192 per week). Assessment of physical activity levels in the EBBA-I subjects is described in greater
193 detail elsewhere (Emaus et al., 2008). Because very few subjects reported high activity, groups
194 3 and 4 were combined in the analyses. In addition to the questionnaires and clinical visits,
195 subjects complete detailed food diaries for seven days across the cycle (days 3-6 and days 21-
196 23), which were then scored by nutritionists at the University of Oslo to determine typical energy
197 intake. Height and weight at baseline were recorded using standard techniques (Furberg et al.,
198 2005) and body mass index (BMI) was then calculated as weight (kg)/ height (m^2).

199
200 *Statistical analysis:*
201

202 All analyses were performed first with SAS Version 9.2 (SAS Institute Inc., Cary, NC, USA) and
203 repeated in R by a second, independent analyst to confirm results (R Version 2.9.0). Because
204 testosterone values are typically non-normally distributed, we fit our models using both the raw

205 and log-transformed testosterone values. The log-transformed data fitted better the linear
206 regression model assumptions which included normality and homoscedasticity of the errors and
207 consequently was used for the current analyses. Our primary model (Model 1) was a linear
208 regression model of log(testosterone) as a function of maternal age, BMI, time of testosterone
209 sample collection, marital status (married or not), testosterone assay type (Immuno1 vs.
210 Elecsys), and age of the youngest child. Age of youngest child was coded with two dummy
211 variables to distinguish three groups: women with no children, women with a child ≤ 3 years and
212 women with children all > 3 years. In a separate model (not shown), we also considered the
213 interaction between marital status and age of youngest child. Because the limit of detection
214 (LOD) of the assay was 1.0 nmol/L, all samples with testosterone levels below the LOD were
215 assigned a value of 1.0 nmol/L for analysis. Sensitivity analyses were subsequently conducted
216 to test whether the results differed when we instead assigned a value of LOD/ $\sqrt{2}$ or LOD/2 to
217 testosterone concentrations below the sensitivity limit.

218

219 A secondary linear regression model (Model 2) was fit which was similar to Model 1 but with
220 adjustment for three additional energetic variables: average daily energy intake, typical physical
221 activity level, and typical hours of sleep per night. Finally a pair of models (Models 3 and 4) was
222 fit for mothers only, analyzing testosterone levels in relation to parity and age of youngest child
223 (treated continuously). Parity was modeled both as strictly ordinal (1-5 children) and as groups
224 (1, 2, or 3+ children) and the second of the two models included the energetic variables. All
225 variables were chosen *a priori* and included in the final models, even if not significant. Model
226 assumptions of linearity between covariates and outcome and normally distributed error with
227 constant variance were checked. For each model, we identified outliers and influential points
228 and reran the models excluding those subjects. All p-values reported are two-tailed, with an
229 alpha level of $p=0.05$.

230

231 **Results:**

232
233 *Demographic measures:*
234

235 Table 1 displays demographic data stratified into three groups based on motherhood status:
236 women with no children, women with a child age ≤ 3 years and women with children all age > 3
237 years. The mean age of the women was 30.9 years (range 25.0-35.9), however, non-mothers
238 were significantly younger than both groups of mothers. Fewer than half of non-mothers were
239 married, moreover, compared to over 80% of mothers. Mothers of young children reported
240 significantly higher average daily energy intake than mothers of older children, but neither group
241 differed significantly from non-mothers. Mothers of young children reported getting significantly
242 fewer hours of sleep on a typical night than non-mothers. The three groups showed no
243 significant differences in height, weight, BMI, age at menarche, physical activity level, or time of
244 serum sample collection.

245
246 *Association of testosterone with marriage and motherhood:*
247

248 Results from our primary model (Model 1) show that after adjusting for covariates, having a child
249 age ≤ 3 is associated with lower testosterone levels and there is a trend towards marriage being
250 associated with lower testosterone levels as well ($p=0.10$) (Table 2). Mothers with a child age
251 ≤ 3 had lower testosterone levels than both women without children ($p=0.02$) and mothers of
252 older children ($p=0.002$; not shown). Reflecting well-documented diurnal rhythms, time of serum
253 sample collection was also a significant predictor of testosterone levels ($p=0.01$). The type of
254 testosterone assay used was a highly significant predictor of testosterone levels, but neither
255 BMI nor age was associated with testosterone levels. In a separate model, the interaction
256 between marital status and age of youngest child (no children, child age ≤ 3 , and child age > 3)
257 was not significant ($p=0.28$ for two degree of freedom test). Figure 1 shows mean (log)
258 testosterone values and 95% confidence limits by marital and motherhood status from this
259 interaction model, after adjustment for age, BMI, type of testosterone assay, and time of sample

260 collection. In our larger model (Model 2), we further adjusted for average daily energy intake,
261 physical activity level, and hours of sleep per night, however none of these variables was a
262 significant predictor of testosterone levels (Table 2).

263
264 *Predictors of testosterone levels among mothers:*

265 In Model 3 we investigated the influence of parity and age of youngest child (entered as a
266 continuous variable) on testosterone concentrations in mothers, further adjusting for marital
267 status, age, BMI, type of testosterone assay, and time of serum sample collection (Table 2).
268 Age of youngest child was highly significantly and positively associated with testosterone levels
269 ($p=0.009$) among the 84 mothers in the sample. Time of sample collection was no longer a
270 significant predictor of testosterone levels. The results were unchanged regardless of whether
271 we considered parity continuously (1-5 children) or by group (1, 2, or 3+ children), and the latter
272 is presented in Table 2. When we further adjusted for the energetic variables (caloric intake,
273 activity level, and total sleep), results were very similar and none of the energetic variables was
274 a significant predictor (Model 4, not shown).

275
276
277 Model assumptions were reasonable when using testosterone on the logarithmic scale. The
278 exclusion of outliers and influential points did not change the estimates for our covariates by
279 more than 5%, therefore all subjects were retained in the models. In sensitivity analyses
280 assigning a testosterone value of $LOD/\sqrt{2}$ (rather than LOD) to samples below the sensitivity
281 limit (not shown), compared to the original models, two differences emerged. First, our
282 estimates for the association between marriage and testosterone concentrations became larger
283 (i.e. less conservative), however due to increased standard errors, our p-values became larger
284 as well. Second, our estimates for the association between having a young child and
285 testosterone concentrations became larger and the p-values became smaller, suggesting that
286 our original estimate and p-values were a conservative assessment of that relationship. These

287 findings were even more pronounced when we reran the sensitivity analyses instead assigning
288 a testosterone value of LOD/2 to samples at the sensitivity limit (not shown).

289
290 **Discussion:**
291

292 We hypothesized that marriage and motherhood would be associated with lower testosterone
293 levels, and that testosterone levels would be particularly low among mothers of young children.
294 In our 195 reproductive-age Norwegian women, we found differences in the predicted direction,
295 namely married women showed a trend towards lower testosterone levels than unmarried
296 women and mothers of children age ≤ 3 had significantly lower testosterone concentrations than
297 women with older children. To our knowledge, the results of the current study are the first of
298 their kind in Western women of older reproductive age and they confirm and extend Kuzawa et
299 al. (2010)'s previous work. Both studies found lower testosterone levels (32% lower
300 testosterone in the Filipino study vs. 14% in the current study) among mothers of young children
301 compared to women who did not have young children, despite considerable differences in
302 culture and sociodemographics. In general, the Filipino women were younger (age 20-22 vs 25-
303 35 for our study population), many did not head their own households, and many were still in
304 school, compared to our older, more established population.

305

306 Our findings also agree with similar research in males, in which fatherhood is frequently, though
307 not always, associated with lower testosterone levels (Gettler et al., 2011a; Gray et al., 2004;
308 Gray et al., 2002; Gray et al., 2006b; Kuzawa et al., 2009; Muller et al., 2009). In males, lower
309 testosterone levels among fathers may be related to direct involvement with the daily care of
310 children and/or the larger societal context. For instance, in two neighboring, traditional
311 Tanzanian populations, one with high levels of direct paternal care (the Hadza), and the other
312 with low levels of direct paternal care (the Datoga), found that only the high-investing Hadza
313 experienced testosterone changes related to paternal status (Muller et al., 2009). Indeed,

314 cross-cultural differences in child-rearing norms may account for mixed findings on the
315 relationship between paternal testosterone levels and the age of the youngest offspring (Gray,
316 2003; Gray et al., 2002; Gray et al., 2006a; Muller et al., 2009) or the amount of direct paternal
317 investment (Gettler et al., 2011a; Gray et al., 2002). Future research on this question in women
318 should more closely examine quantity and quality of direct parental care in relation to maternal
319 testosterone levels, particularly longitudinally.

320

321 Evolutionarily, the low testosterone levels found in parents of young children may represent a
322 trade-off from investment in mating effort to investment in parenting effort. While testosterone
323 facilitates intra-sexual competition for mates, libido, and aggression, those behaviors may be
324 unnecessary for, or even incompatible with, effective parenting, and thus there may have been
325 natural selection for lowered testosterone levels coincident with increased parental investment
326 (Gray and Campbell, 2009). Alternatively, low testosterone levels in parents of young children
327 could be a by-product of the high energetic demands of caring for a small child, given that some
328 types of acute or chronic stress may be associated with reduced testosterone levels (Bribiescas,
329 2001). To address this possibility, we included average energy intake, physical activity level,
330 and hours of sleep per night in a larger model, however none of these variables predicted
331 testosterone levels. Other studies have similarly failed to identify energetic variables as
332 important mediators in the relationship between marriage, parenting, and testosterone (Gettler
333 et al., 2011b; Gray et al., 2002; Kuzawa et al., 2010), thus we suggest that the low testosterone
334 levels seen in mothers of young children are unlikely to be attributable to energetic stressors.

335

336 One strength of our study is that unlike previous work, we used multivariable modeling to
337 examine how the interaction between marital and parental status was associated with
338 testosterone levels. Among childless women and mothers of older children, marriage tended to
339 be associated with lower testosterone levels. Interestingly, among mothers of young children,

340 the opposite pattern was present, whereby marriage was associated with higher testosterone
341 levels. Although the sample size for that sub-population was small and the results were not
342 statistically significant, it suggests that in those women, there may be an extreme trade-off
343 between intense investment in parental care and minimal investment in mating effort. Because
344 human children are so altricial and our species' evolved family structures tend to facilitate
345 biparental care, single mothers of young children represent an evolutionarily novel situation.
346 Notably, single mothers of older children did not have lower testosterone levels, possibly
347 indicating that once dependent care becomes less demanding, single mothers can again invest
348 in mating effort. It is also possible that elevated psychosocial stress commonly reported among
349 single mothers (Avison et al., 2007; Franz et al., 2003) may manifest itself in physiological
350 changes that reduce testosterone and other markers of mating effort, though we know of no
351 research that directly addresses this question.
352
353 Another notable strength of our study was our inclusion criteria. In Kuzawa et al. (2010), there
354 was cross-subject variation as to when, during the menstrual cycle, testosterone concentrations
355 were measured, and some subjects were using oral contraceptives or breast-feeding at the time
356 of participation. Although the authors considered these important covariates during analysis, our
357 study design reduced or entirely eliminated these potential sources of variation in testosterone
358 levels. Because the original EBBA-I study was designed to look at natural variation in hormone
359 levels across the cycle, women were excluded if they had been pregnant or breast-feeding
360 within the last six months. Similarly, because subjects were ineligible if they had used hormonal
361 contraception within the previous six months and all serum samples were taken within a small
362 window of the follicular phase of the menstrual cycle, thus we can rule out those variables as
363 potential confounders in our analyses.
364

365 Issues with the testosterone assays used present several limitations for our study, unfortunately.
366 First, our testosterone level were measured in serum, rather than saliva, as in many related
367 studies (Gray et al., 2002; Kuzawa et al., 2010) and thus reflect free (biologically active)
368 hormone levels as well as the bound, inactive fraction. Ideally, we would prefer to examine both
369 the free and the total levels, as can be done with current assay technologies, however it is worth
370 noting that we found the same relationships with total serum testosterone that have previously
371 been found in studies analyzing salivary (free) testosterone and previous work has found similar
372 relationships between testosterone and fatherhood regardless of whether testosterone was
373 measured in saliva or serum (Kuzawa et al., 2009). ▼

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374
375 Second, the high LOD of the testosterone assay (1.0 nmol/L) meant that the range of values
376 below 1.0 nmol/L was truncated and all women with testosterone levels at or below that level
377 were assigned a value of 1.0 nmol/L (which actually represents the highest possible
378 concentration that they could have). Because women with young children were
379 disproportionately represented among subjects with testosterone levels at the LOD, it is likely
380 that their group mean is even lower, suggesting that our findings may actually be an
381 underestimate of the true relationship between having a young child and testosterone levels.
382 When we conducted sensitivity analyses assigning $\text{LOD}/\sqrt{2}$ or $\text{LOD}/2$ (rather than simply the
383 LOD, 1.0 nmol/L) to samples with values below the LOD, we found that the estimate of the
384 association between marriage and testosterone grew stronger, but the p-value increased due to
385 increased standard errors. The association between having a young child and testosterone
386 levels similarly strengthened and became more significant, suggesting that we are likely
387 underestimating the strength of the relationship. Therefore, we predict that with the more
388 sensitive assays available today, we would see an even greater effect size between
389 testosterone levels and having a young child. Finally, the interpretation of our results is
390 complicated by the fact that the clinical laboratory switched from the use of one testosterone

391 assay to a second one partway through the study. Although the correlation of testosterone
392 levels measured in samples run in parallel on the two assays was high (particularly among high
393 testosterone samples), it still represents an additional source of error and variation.
394 Nevertheless, as shown, there were still associations between testosterone and motherhood
395 after adjusting for testosterone assay type in our models.

396

397 In our study, one potential source of misclassification bias is based on marital status. First, our
398 questionnaire did not differentiate between married and *samboer*, a legally recognized
399 cohabitation status which is best translated as “living as married”. Unlike in some Western
400 nations, in Norway, there is no stigma to this designation and it is common for *samboer* couples
401 to have children and never formally wed. Based on that, we considered women who were
402 married or *samboer* as a single group, indicative of a highly committed relationship. If women
403 who were *samboer* were actually more similar to unmarried women, their exposure
404 misclassification would likely have been non-differential and biased our results towards the null.
405 In addition, we do not have data on sexual activity or sexual fidelity in any of the women studied
406 (married or single), although results from other studies suggest they may be useful to consider
407 in relation to testosterone concentrations in women (van Anders et al., 2007). Additional
408 research is needed to disentangle the associations between testosterone, relationship status,
409 and sexual activity in women.

410

411 Because our study was cross-sectional, we cannot determine the direction of causality or reject
412 the possibility that women with high testosterone levels are less likely to marry and become
413 mothers. Certainly, some clinical conditions characterized by supranormal testosterone levels,
414 such as polycystic ovary syndrome, may be associated with lower fecundity (Mellembakken et
415 al., 2011), however our inclusion criteria excluded women likely to have a hormonal or serious
416 medical condition. To our knowledge, no study has examined whether testosterone levels in

417 women within the normal range are related to their likelihood of marrying or conceiving. Our
418 finding, furthermore, that testosterone levels were only lower in mothers of young children
419 suggests that the suppression is temporary ("state", not "trait") and that as children age beyond
420 their peak dependent years, a mother's testosterone levels may increase.

421

422 Our findings agree with and extend previous research in females and the larger body of work in
423 males. Nevertheless, it remains uncertain as to whether the associations between testosterone,
424 marriage, and parenting in females represent adaptations shaped by natural selection (as has
425 been argued persuasively in males) or whether there are alternative explanations. Limited
426 research in other species has found that high testosterone levels may be associated with
427 territorial defense by females, but reduced care of offspring, supporting an adaptive explanation
428 (Fite et al., 2005; Moller et al., 2005). It is also possible that this relationship in females emerged
429 as a by-product of selection for testosterone-mediated modulation of mating and parenting effort
430 in males, although testosterone levels co-vary in males and females in some, but not all,
431 species (Ketterson et al., 2005).

432

433 The fact that testosterone production in the two sexes differs further complicates the
434 comparison. In males, the testes produce the majority of testosterone with negligible adrenal
435 contributions, whereas in females, the ovaries and adrenals contribute approximately equally
436 (Longcope, 1986). Thus, in men, differences in testosterone associated with pair-bonding and
437 fatherhood are presumed to be related to down-regulation of testosterone production by the
438 testes (Kuzawa et al., 2009). While it may be most obvious to attribute differences in circulating
439 testosterone levels among females to homologous differences in ovarian function, differences in
440 adrenal androgen production and activity may also be implicated. A comparison of, luteinizing
441 hormone (LH) and adrenocorticotrophic hormone (ACTH) concentrations in relation to marriage
442 and motherhood might help to resolve, this question. If levels of LH, but not ACTH, differ by

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443 marital or motherhood status, it would provide evidence for differences in gonadal testosterone
444 production, possibly as a by-product of selection in males. Conversely, differences in ACTH
445 would implicate differences in adrenal androgen production, possibly indicating an adaptive
446 neurobiological response to pair-bonding and parenting. Acute variation in ACTH concentrations
447 in relation to stress and the pulsatile nature of ACTH release may make such a comparison
448 difficult or impractical, however (Carnes et al., 1988; Lennartsson et al., 2012; Van Cauter et al.,
449 1981). It may be similarly important to consider the role that psychological mechanisms play in
450 modulating the relationship between testosterone, marriage, and motherhood in females.
451 Several studies have examined maternal behaviors in relation to testosterone, finding that
452 among new mothers, higher testosterone is associated with less affectionate behavior towards
453 their infants (Fleming et al., 1997), more mood disturbances (Buckwalter et al., 1999), and
454 higher scores on depression and anger inventories (Hohlagschwandtner et al., 2001). In young
455 women, furthermore testosterone administration increased neural response to (non-related)
456 infant crying (Bos et al., 2010).

457

458 Finally, we cannot rule out the role that other hormones involved in the co-regulation of female
459 reproductive function may play in driving this relationship. It will be important for future research
460 to consider not only the associations between testosterone, marriage, and motherhood, but also
461 the role of other hormones implicated in mating and nurturing behavior, such as oxytocin
462 (Feldman, 2012; Feldman et al., 2007; Gordon et al., 2010; van Anders et al., 2011), cortisol
463 (Fleming et al., 1997; van Bakel and Riksen-Walraven, 2008), and the ovarian steroids,
464 estradiol and progesterone (Fleming et al., 1997; Rosenblatt, 1994). Indeed, given that much
465 ovarian testosterone is aromatized to estrogen, it is possible that the upregulation of
466 testosterone production associated with mating effort is, in fact, due to selection for increased
467 estrogen production. Similarly, the lower testosterone we have found among women with young
468 children could represent selection for temporary reproductive suppression through decreased

469 estrogen levels. This possibility may be further explored by examining estrogen levels in relation
470 to maternal status.

471

472 In conclusion, our study showed that among Norwegian women, both marriage and
473 motherhood were associated with lower testosterone levels and that having a child age ≤ 3 was
474 a particularly strong predictor of low testosterone levels. Future work should address
475 longitudinal changes in female testosterone levels over time, the biological mechanisms
476 underlying the relationships, and the association between testosterone and quantity and quality
477 of direct maternal care.

478

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706 **Table and Figure Captions:**

707
708 Table 1. Characteristics of the study population (mean±S.D.).

709
710 Table 2. Multiple regression models predicting testosterone levels. Testosterone data were
711 log-transformed for the regression, thus units are expressed as log(nmol/L) with 95% CI
712 indicated. Results in bold indicate significance at $\alpha=0.05$.

713
714 Figure 1. Comparison of mean log(testosterone) levels in log(nmol/L) by marital and
715 motherhood status. The error bars show the upper 95% confidence interval for the predicted
716 response among subjects with the Immuno1 testosterone assay and the mean values of BMI,
717 age, and time of serum sample collection.

718
719 Figure 2. Relationship between log(testosterone) and age of youngest child. Data points are
720 observed values. The lines show the slope and a 95% confidence interval for the slope from the
721 regression model adjusted for marital status, time of serum sample collection, body mass index,
722 age, parity, and testosterone assay type.